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Crocomo, O.J. and L.C.Basso. Plant Biochemistry Sector,Centro de Energia Nuclear na Agricultura, CNEN, USP, Piracicaba,S.P., Brasil. AMINO ACID METABOLISM IN RELATION TO THE POTASSIUM STATUS IN SESAMUM.

The metabolism of the amino acids ornithine, citrulline and arginine, all labelled with ^{14}C, was studied in intact plants and in leaf homogenates of Sesamum indicum L. growing in Hoagland and Arnon's complete and K-deficient nutrient solutions. These amino acids produced the corresponding ^{14}C-amines putrescine, N-carbamylputrescine (NCP) and agmatine. Putrescine was the amine having the highest concentration,NCP intermediate, and agmatine the lowest. The distribution of the radioactivity among the amino acids of the ornithine-urea cycle was greatly affected when potassium was depleted. The conversions of arginine t. ornithine, and of citrulline to arginine decreased in -K, while the reaction ornithine to citrulline increased. This led to an accumulation of arginine and citrulline.

The data obtained permits the conclusion that not only is there direct decarboxylation of these amino acids in -K, but also that there is an interconversion among the amines; this is supported by the fact that labelled putrescine arises from ^{14}C-arginine.

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Boggess, S. F. and A. W. Naylor, Department of Botany, Duke University, Durham, N. Carolina 27706. PARTIAL PURIFICATION AND PROPERTIES OF ORNITHINE TRANSCARBAMOYLASE FROM NOSTOC MUSCORUM KÜTZING.

Sonic extracts of algal cells were fractionated with streptoycin, heat, salt, adsorbents, ion exchange cellulose and gel filtration; preparations 500-600 fold purified were unstable, therefore, 150-250 fold purified preparations were used for characterization studies. Competitive inhibition occurs with phosphate, sulfhydryl reagents and azide. Activity was lost on dialysis. Michaelis constants were determined as 0.7 millimolar for carbamoyl phosphate, and about 2.5 mM for ornithine. pH 9.5 was optimum for citrulline synthesis. Energy of activation was 12.3 kilocalories per mole. The molecular weight is about 75,000. A selection of metabolites which might conceivably be regulators of the enzyme were tested for effect on enzyme activity; none were found to alter the velocity of citrulline synthesis more than slightly. These facts along with classical kinetics indicate that Nostoc ornithine transcarbamoylase is not not subject to allosteric regulation by metabolites.

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Widholm, J. M., Department of Agronomy, University of Illinois, Urbana, Illinois, 61801. TOBACCO AND CARROT CELL CULTURES RESISTANT TO GROWTH INHIBITION BY A TRYPTOPHAN ANALOG.

A tryptophan analog, DL-5-methyltryptophan (5MT), inhibits tobacco and carrot cell culture growth completely when present in the liquid medium (10 and 50 mg/l, respectively). The inhibition is apparently caused by false feedback inhibition of anthranilate synthetase, the control enzyme in tryptophan biosynthesis. This is indicated by the reversal of the 5MT growth inhibition by anthranilate, indole or tryptophan. Also 5MT and tryptophan potently inhibit the enzyme.

After a period of time some tobacco and carrot cells begin to grow in the growth inhibitory 5MT medium. These cells are thereafter resistant to the analog. The anthranilate synthetase from these resistant cultures is more resistant to inhibition by both tryptophan and 5 MT indicating that this more resistant enzyme causes the resistance.

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Chen, Shepley S. C. Dept. of Biological Sciences, University of Illinois at Chicago Circle, Chicago Illinois 60680 METABOLIC CHANGES IN DORMANT SEEDS DURING DRY STORAGE.

Dormant Avena fatua seeds were dehulled, surface-sterilized in pure isopropanol, or in 1% NaClO, washed and air-dired. These dry seeds are exposed to C-14 ethanol vapor in a sealed glass tube, and incubated for a period of time. They are then homogenized and extracted in 80% ethanol. Alcohol-soluble fraction was chromatographed on ion exchange resins. The result showed that an appreciable amount of radioactivity was taken up and incorporated into sugars, amino acids, and organic acids. Labeled amino acids and sugars were released from insoluble residue upon acid hydrolysis. Some of the radioactivity associated with the trichloroacetic acid-insoluble material could be released by treatment with pronase, suggesting that protein might have been synthesized.

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Morris, R.O., L.A. Norris and G.B. Jacobsen. Department of Agricultural Chemistry, Oregon State University, Corvallis, Oregon 97331. THE APPEARANCE OF PROTEINS ASSOCIATED WITH PROLIFERATING PLANT TISSUE.

Variation in acrylamide gel electrophoretograms derived from the soluble fraction of proliferating or fully differentiated pea root tissues has been noted (Morris, BBA, 127, 273(1966). We now report details of the variation.

Four bands (designated D_{1-4} having mobilities 0.39, 0.71, 0.74 and 0.76 with respect to the dye front) are markedly increased in roots undergoing lateral proliferation as a response to the application of 2,4-dichlorophenoxyacetic acid. The response of the bands to protein-specific stains and their sensitivity to proteolytic enzymes indicates that they are proteins. One band, D_2, contains a peroxidase as evidenced by both guiaicol and benzidine stain. A greater than fifty-fold purification of these proteins has been achieved by ammonium sulfate fractionation and batch absorption onto CM-cellulose. As yet, insufficient amounts are available for characterization, however, their distribution within a normal root and the correlation of incorporation of precursor amino acid into D_1 with cytological evidence of initiation of cell division suggests that these proteins are related in some way to the proliferation process.

(Supported in part by the Herman Frasch Foundation).

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Aung, L. H. Department of Horticulture, Virginia Polytechnic Institute & State University, Blacksburg, Virginia 24060. THE LOCALIZATION OF THE SITES AND NATURE OF ROOT-FORMING SUBSTANCES IN TOMATO SEEDLING.

Determination of the organ sites and chemical nature of root-forming substances in Lycopersicon esculentum Mill. seedlings was made. Results of organ excision indicated the young cotyledons were the primary site of root-forming and root-inhibiting substances. As the cotyledon matured the activity of these substances decreased and greater activity was found in the developing plumular leaves. Application of 2,3,5-triiodobenzoic acid (TIBA) at 10^{-3} or 10^{-4}M in lanolin to the hypocotyl below the cotyledonary node significantly prevented root formation and to a lesser degree if applied to the petioles. Bioassay, using a new tomato rooting test, of the neutral, acidic and bound fractions of the cotyledon extracts after paper chromatography showed biological activities in all three fractions. The major regions of root promotion were located between R_f 0.3-0.5 and with root-inhibiting regions near the origin and end of the chromatograms. Gas-liquid chromatography of selected active regions of the paper chromatogram resulted in a partial identification of indolepropionic and indolebutyric acids in the extracts. The combined evidence indicated that the root-forming substances in tomato seedlings resemble auxin compounds.

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Haissig, Bruce E. U.S.D.A. Forest Service, Institute of Forest Genetics, Rhinelander, Wis. 54501. ENZYME ACTIVITY CHANGES DURING ADVENTITIOUS ROOT INITIATION.

Cuttings were made from bean (Phaseolus vulgaris) plants with 4 to 5 cm long primary leaves, either by severing the hypocotyl at and 5 cm below the cotyledons (DC), or by removing cotyledons and severing the hypocotyl 5 cm below the cotyledonary node (LC). Cuttings were then treated with 0.025M MES-NaOH buffer, pH 6.0 (DC and LC), or with this buffer containing 5 x 10^{-5}M indole-3-acetic acid (LC + IAA). Every 24 hours thereafter, the cuttings were supplied with fresh distilled water. The number of roots per cutting increased by treatment in the order DC, LC, LC + IAA; primordia first appeared by reverse order of treatment. The activities of glucose-6-phosphate dehydrogenase (G-6-PD), glyceraldehyde-3-phosphate dehydrogenase (G-3-PD), and citrate synthase (CS) were determined for extracts of basal hypocotyl segments harvested 0, 24, 48, 72, and 96 hours after treatment. The largest changes noted during root regeneration were for G-3-PD (NAD), G-6-PD, CS, and G-3-PD (NADP), respectively. Activity increases preceded primordium initiation, and the total activity change for each enzyme related to the number of primordia formed. Thus, the earliest and greatest increases in enzyme activity were found in IAA-treated cuttings.

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Gordon, S. A. and E. M. Buess. Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Ill. 60439. ROOT EMERGENCE AND AUXIN-RIBONUCLEIC ACID INTERACTIONS IN COLEUS LEAVES.

Exposure of the lamina of the isolated Coleus leaf to γ radiation retards the emergence of adventitious roots at the petiole base. This correlative response can be related to decreases in the petiole of auxin, RNA, and protein, and a rise in gross ribonuclease activity 3 days before histogenesis. Supplying auxin to the petiole increases, within 1 day, the levels of RNA, protein, and the number of roots subsequently emerging, and reverses the radiation-induced decrease in RNA and root number. Yet exogenous auxin enhances ribonuclease activity and does so synergistically with radiation. In interpreting experimentally this apparent inconsistency, we will consider: 1) the possibility of more than one petiolar ribonuclease differing in concentration and reactivity; 2) a two-hormone hypothesis that a kinin mediates the effect of auxin on RNA levels; 3) that the increase in RNA induced by auxin is a consequence of a differential activation of hydrolysis and synthesis. Work supported by the United States Atomic Energy Commission.

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Dunkle, L.D. and J.L. Van Etten. Plant Pathology Department, University of Nebraska, Lincoln, 68503. CHARACTERISTICS OF DNA DURING FUNGAL SPORE GERMINATION.

Potential modifications of DNA during fungal spore germination were investigated by determining the physical properties of DNA isolated from non-germinated and germinated spores of Rhizopus stolonifer and Botryodiplodia theobromae. The GC contents were determined by CsCl equilibrium density centrifugation, thermal denaturation, and spectral properties. Sedimentation characteristics were determined by centrifugation through linear-log sucrose density gradients. The DNA from R. stolonifer had a GC content of 38 mole %, a molecular weight of 3.5×10^6 daltons (15S), a compositional heterogeneity of 7.3 mole % GC, and exhibited 42% hyperchromicity upon thermal denaturation in 1X SSC. The DNA from B. theobromae had a GC content of 53 mole %, a MW of 4×10^6 daltons (15.5S), a compositional heterogeneity of 8.5 mole % GC, and 37% hyperchromicity in 0.1X SSC. Spores of both fungi synthesized DNA and incorporated adenosine-8-C^{14} into DNA at some point during the germination period, but the gross chemical characteristics of non-germinated spore DNA were indistinguishable from those of germinated spore DNA. Thus, unlike the DNA of germinating wheat embryos (Chen and Osborne, Nature 225:336. 1970), there are no apparent modifications of the DNA during fungal spore germination.

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Towill, Leigh E. & L. D. Nooden, Botany Dept., Univ. of Michigan, Ann Arbor, 48104. COMPARISON OF HISTONES FROM DIFFERENT ORGANS OF MAIZE SEEDLINGS.

Because of their importance in regulating gene expression, considerable effort has been made to study the histones. It is important to know if the histones of chromatins from different organs differ, since the genes expressed are presumably different. Chromatin has been isolated & purified from corn seedling root, mesocotyl & epicotyl nuclei. Histones were extracted from the chromatin with 0.4 N H_2SO_4 & electrophoresed in urea-acrylamide gels. The stained histone bands were measured quantitatively with a densitometer. Our results show both a qualitative & quantitative similarity for the histone patterns from all 3 organs. After mercaptoethanol treatment, 4 major bands exist & have been tentatively identified as histone I, IIa, IIb-III & IV. Mild oxidation of the histones leads to the formation of a fifth major band, probably an aggregate of histone III. The relative amounts from each organ are approximately: 17% histone I, 44% histone IIa, 22% histone IIb + III, & 17% histone IV. Previous reports suggest that the histones of different plant organs may differ qualitatively in some cases but not in others. We have confirmed the findings of Fambrough et al (1968) on pea seedlings in that different vegetative organs contain the same histones; however, we find no quantitative differences.

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Borchert, R., Botany Department, University of Kansas, Lawrence, Kansas 66044. INDUCTION OF DNA - SYNTHESIS DURING WOUND HEALING OF TUBER TISSUE.

Cutting of potato tuber tissue induces the following processes: 1. suberization of the cell walls adjacent to the wound, 2. rapid increase in the rate of respiration and other metabolic activities in all tissues down to 1 to 2 mm below the wound due to enzyme induction, 3. onset of DNA-synthesis after a lag period of 8 h in one or two subperipheral cell layers. Analysis of the induction of DNA-synthesis requires the experimental separation of the various component processes of wound healing. This has been achieved, in part, by the following procedures: 1. mechanical separation of the peripheral and subperipheral layers from the tissue below, 2. induction of DNA-synthesis in previously activated tissue by recutting 0.5 mm below the original cut, 3. use of inhibitors that specifically interfere with DNA-synthesis but not with protein and RNA-synthesis, 4. use of tubers stored more than one year in which the capacity for metabolic activation is fully preserved, but that for cell division - and preceeding DNA-synthesis - has been lost.

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Beasley, C. A. and Irwin P. Ting. Biology Department, University of California, Riverside, California 92502. FIBER AND EMBRYO DEVELOPMENT FROM COTTON OVULES CULTURED IN VITRO.

Fertilized ovules of cotton were aseptically cultured on the surface of liquid medium. Such ovules, when collected from actively flowering parent plants, and treated with gibberellic acid, produced fibers to nearly the extent of those left on the plant. Gibberellic acid markedly stimulated the extent of fiber growth, and overcame the inhibitory effect of abscisic acid. Normal embryos were produced within some of the cultured ovules. Occasionally embryo development occurred at the expense of fiber growth, and the epidermis of the ovule produced callus only. Ovule collection and culture procedures, quantitative methodology, and the effects of various plant growth regulations will be discussed. Supported by Cotton Incorporated.

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Helgeson, J. P., J. D. Kemp, D. P. Maxwell and G. T. Haberlach. Pioneering Research Lab., Plant Science Research Div., ARS, USDA, Dept. of Plant Pathology, University of Wisconsin, Madison, Wis. 53706. DIFFERENTIAL GROWTH OF PHYTOPHTHORA PARASITICA VAR. NICOTIANAE ON TISSUE CULTURES FROM RESISTANT AND SUSCEPTIBLE TOBACCO PLANTS.

Tobacco tissue cultures derived from a plant that is resistant to infection by race 0 of Phytophthora parasitica var. nicotianae, the causal agent of black shank of tobacco, appear to be colonized at a considerably slower rate and to a lesser degree than tissue cultures derived from a nearly isogenic susceptible plant. Light is not necessary for obtaining the differential. No difference in colonization of the two lines of tissue cultures is seen with race 1 of the pathogen, a race to which both intact plant lines are equally susceptible. The degree of colonization of cultures from both resistant and susceptible plants appears greater at 28 C than at 32, 24, 20 or 16 C. Our preliminary results indicate that the degree of tissue colonization differs with different cytokinin and auxin-controlled morphological forms of tissue cultures. As a working hypothesis, we consider that this differential colonization of tissue cultures from resistant and susceptible plants is due to the expression, in tissue cultures, of the resistance mechanism seen in the intact plant.

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Comer, A. E., Department of Botany, University of Minnesota, Minneapolis, Minnesota 55455. XYLOGENESIS IN ISOLATED COLEUS PITH EXPLANTS.

An experimental system, homogeneous in cell type, has been investigated for use in the study of wound-vessel member (WVM) induction. Longitudinal pith slices were explanted from the second internode of single axis Coleus plants. These explants were cultured on sucrose agar for ten days or less. Auxin (indoleacetic acid) was found to be an absolute requirement for WVM induction. Benzyl-adenine (0.01 to 3.0 ppm) did not stimulate WVM formation alone or when in combination with auxin. Pre-existing vascular tissue is not necessary for WVM induction. It is significant that the time course of WVM appearance in this system is comparable to that of wounded intact Coleus plants.

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LaMotte, C. E. and Myrna A. Whigham. Department of Botany & Plant Pathology, Iowa State University, Ames, Iowa 50010. FACTORS AFFECTING VASCULAR REGENERATION IN AUXIN-TREATED, EXCISED INTERNODES OF COLEUS.

Excised no. 5 internodes of Coleus blumei, wounded in one side and supplied apically with 1% IAA in lanolin, regenerated less phloem and sometimes less xylem than comparable internodes in intact plants (unlike results reported by Thompson 1965 & 1967 and Thompson and Jacobs 1966). To determine limiting factors for regeneration in such IAA treated internodes, 2 cytokinins and GA_3 were applied, singly and in combinations, and treatments designed to improve their nutrition and water status were tried. Kinetin at 0.01% in lanolin stimulated phloem regeneration to a small extent and GA at 0.1, 0.5 and 1% inhibited both xylem and phloem regeneration. Weak sunlight (80 - 200 ft-c) stimulated xylem regeneration only. Sucrose (2% in agar) stimulated phloem and, perhaps, xylem regeneration in strongly illuminated internodes. When "explants" each consisting of a no. 6 leaf pair and its subtending internode were wounded, kept turgid and daily illuminated, they regenerated about twice as much xylem and nearly as much phloem as internodes in intact plants. Speculation regarding the bases for differences between our findings and earlier ones will be presented.

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Benson, A. A., and Richard F. Lee. Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92037 THE SULFOCARBOHYDRATE METABOLIC PATHWAY IN PLANTS.

The plant sulfolipid, 6-deoxy-6-sulfo-α-D-glucosyl-2,3-D-diglyceride, is a major chloroplast lipid. It occurs in other tissues such as sugar beet root in even larger relative amounts. While its biosynthesis is not yet understood its catabolism is now apparent. Sulfolipase, readily activated in leaves or algae, deacylates the lipid to form glyceryl sulfoquinovoside, GSQ. Sulfolipase activity was inhibited by GSQ. A very active sulfolipase has been prepared from extracts of Tetrahymena pyriformis. In leaves GSQ appears to be cleaved by phosphorolysis yielding phosphosulfosugar analogs of the glycolytic intermediates. Sulfolactaldehyde, analog of triose phosphate, and sulfolactic acid, analog of phosphoglyceric acid, are always present in plant systems. Sulfopropanediol, the glycerophosphate analog, a major component of all diatom algae, is readily observed in plant tissues. The final degradation product, sulfoacetic acid, is readily obtained from GSQ in leaves of Erythrina crista-galli, the coral tree, which is known to accumulate sulfoacetylated alkaloids. Leaves of alfalfa, Medicago sativa, produced sulfolactic acid as the major product. Diatoms and Anacystis nidulans reduced sulfolactaldehyde to sulfopropanediol. Final degradation of sulfoacetic acid to sulfate and glyoxylate does occur in plants but sulfate is produced slowly and is rarely a major product of GSQ metabolism as has been observed in certain bacterial strains. This work was supported by NSF Grant GB 15500

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Monica Lik-Shing Tsang, Eliezer E. Goldschmidt, and Jerome A. Schiff, Biology Dept., Brandeis University, Waltham, Mass. 02154. ADENOSINE 5' PHOSPHOSULFATE (APS^{35}) AS AN INTERMEDIATE IN THE CONVERSION OF ADENOSINE 3' PHOSPHATE 5' PHOSPHOSULFATE ($PAPS^{35}$) TO ACID-VOLATILE RADIOACTIVITY.

An intermediate, identified as APS, has been found in the enzymatic conversion of $PAPS^{35}$ to acid-volatile radioactivity by enzyme fractions "A" and "S" from Chlorella pyrenoidosa. "A" was found to be a Mg^{2+}-dependent 3' nucleotidase specific for 3'5' diphosphonucleotides which converts PAPS to APS. Authentic APS^{35} is a substrate for "S" and yields acid-volatile radioactivity in the presence of Mg^{2+} and a thiol. This is of some interest since Chlorella is an assimilatory sulfate reducer and APS has been reported as a substrate only for dissimilatory sulfate reduction. PAPS production is undoubtedly concerned with providing a source of active sulfate for esterification reactions, but since fraction "A" can convert PAPS to APS, this may be yet another mechanism for forming APS despite the unfavorable equilibrium of the ATP sulfurylase reaction. The acid-volatile radioactivity in these experiments seems to be due to sulfite; whether there are two separate systems forming thiosulfate and sulfite or whether these are alternative products of the same system under different conditions remains to be determined.

NSF (GB4231, GB25920)sup.

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Asen, S. Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705. FACTORS AFFECTING THE FORMATION OF ANTHOCYANIN-FLAVONOL CO-PIGMENT COMPLEXES AND THEIR IMPORTANCE ON FLOWER COLOR.

The phenomenon of co-pigmentation, the complexing of anthocyanins with flavonols, resulted in an increase in extinction as well as a bluing of the anthocyanin color. The increase in extinction and the color change were directly related to pH, concentration of anthocyanin, and the molar ratio of anthocyanin to flavonol. Only anthocyanins with an ortho-dihydroxyl system form a colored metal complex but in contrast co-pigmentation occurred with glycosides of all six of the major anthocyanidins. Co-pigmentation explains the great variations and the intense color of flowers in a pH range where anthocyanins per se are virtually colorless.